STUDIES ON DORMANCY OF SEEDS OF THE AMERICAN HIGHBUSH CRANBERRY, VIBURNUM TRILOBUM, Marsh.

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ABSTRACT

Dormancy was encountered in both seeds and seedlings of the American highbush cranberry, Viburnum trilobum, Marsh. When seeds were exposed to germinative conditions at 20° C., the temperature usually prescribed for germination of Viburnum species, dormancy was expressed by slow growth of the radicle and the hypocotyl in some seeds, and by what appeared to be complete failure to germinate in others. This type of dormancy was found to be associated with the presence of a water-soluble inhibitor found in both endocarp and seed, as well as with a need for an appropriate temperature treatment. Seedling or epicotyl dormancy was shown by a failure of the shoot to grow following seedling emergence. Dormancy of this type was found to be associated with the presence of the cotyledons. When these structures were completely removed from the seedling, growth of the epicotyl proceeded in a normal manner.

UNIVERSITY OF ALBERTA

STUDIES ON DORMANCY OF SEEDS OF THE AMERICAN HIGHBUSH CRANBERRY, VIBURNUM TRILOBUM, Marsh.

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INTRODUCTION

This investigation is concerned with the nature of dormancy in the seeds of the American highbush cranberry, Viburnum trilobum, Marsh., a species which is commonly said to have a "two-year seed," since the seedling does not produce an actively growing shoot until the second year after planting if the seed is sown out-of-doors (14). Under controlled conditions, the seed will germinate slowly at a temperature of 20° C., but appears to require further exposure to moist conditions at 5° C. in order to effect shoot production.

The catabolic processes of most dormant seeds are favored by temperatures between 2° and 5° C.; therefore, the response of seeds of V. trilobum to 20° C. for germination suggests dormancy of a special type. Failure of the shoot to grow following germination complicates the problem further, and suggests that the epicotyl is in some way prevented from immediate development.

The objectives of the study, therefore, are twofold. They are concerned with the nature of dormancy in the primary root, and with the factor or factors responsible for the expression of dormancy in the epicotyl of the seedling. A knowledge of these factors would be of particular value to plant breeders and to nurserymen working with this species.

LITERATURE REVIEW

Germination and After-ripening

Toole et al. (31) considered germination as a process controlled by a series of interlocking reactions which must be kept in proper balance; the balance being so exact as to be attained only within narrow temperature limits with some seeds. Should a seed require only imbibition of water for germination, the system of reactions is complete and balanced. This implies the presence of readily available reserves within the developing radicle or its contiguous cells, the utilization of which is mediated by pre-existing enzymes. Synthesis of enzymes for the utilization of the main seed reserves of the endosperm or cotyledons is not essential to these early steps, but follows closely. This statement emphasizes that germination is a process antecedent to the use of the major food reserves which these authors considered to be required for seedling growth.

In the case of seeds in which delayed germination is due to failure of the embryo to respond when optimal conditions for germination are present, Eckerson (10) speaks of a need for an "after-ripening period." "After ripening," she (10) states, "refers to those treatments which result in necessary changes taking place in the embryo prior to germination." Most

present-day workers would agree with this, although Barton (5), Crocker (9), Giersbach (16), and others, would have extended the definition to include those changes which must take place within the embryo before full growth of the seedling is attained. In other words, in the case of seeds possessing dormant epicotyls following germination, the term "after-ripening" would also apply to those changes which must take place in this part of the embryo prior to full growth of the seedling.

The term "after-ripening" has been used rather loosely in some present-day literature to refer to those processes which result in changes in the seed-coat or fruit-coat. Such changes, while often necessary for germination, generally precede "after-ripening."

Eckerson (10) studied changes occurring in Crataegus embryos during after-ripening. Following exposure to 5° C. for 80 - 90 days, fat breakdown was observed and sugar appeared. Accompanying these changes was a slow but constant increase in acidity and enzyme activity. Rapid metabolism of fats did not occur until the acidity reached a certain level. Deleano, according to Eckerson (10), observed identical changes in the embryos of Ricinus communis, L., although the time required for germination was far shorter.

Pack (22), working with the seeds of Juniperus virginiana, L., noticed a similar increase in H^+ ion concentration of the embryo up until the time of germination. He also

noted changes in the endosperm accompanying after-ripening. Fat breakdown in these tissues took place most rapidly in the region adjoining the hypocotyl, and this was accompanied by the first notable increase in sugar. Steady increases in amino nitrogen were also apparent up until the time of germination.

Pack (22) considered the breakdown of endosperm material of major importance in the after-ripening of Juniperus. This is contrary to the observations of Eckerson (10) that after-ripening effects the breakdown of endosperm material. Pack's observations that changes in endosperm fats occur most rapidly in cells near the hypocotyl may suggest, however, that metabolism in the endosperm is engendered by the embryo. Pack (22) stated that the digestion of storage fats and proteins was accompanied by the synthesis of many formative and metabolic compounds. He considered that the action of low temperature in retarding respiration reduced "combustion" to a minimum, thereby favoring the accumulation of formative materials in the tissues of the endosperm. "This accumulation of cell-building or cell-active materials, together with the activation of enzymes," he stated, "probably leads to the after-ripening of dormant organs."

While Pack's conclusions (22) regarding changes in the endosperm of Juniperus may be quite sound, his failure to deal with concurrent changes in the embryo leaves his theory without the support of experimental evidence.

Stokes' work (25, 26, 27) with the seed of cow parsnip, Heracleum sphondylium, L., however, provides some support for Pack's deductions. In dormant Heracleum seed, the embryo is a minute ball of cells. At 15° C., growth of the embryo does not take place, but at 2° C. the growth is normal. Accompanying this growth is a rapid breakdown of food reserves, particularly proteins in the endosperm, with rapid transfer to the cells of the embryo. At 15° C., breakdown of proteins also occurs, though it is not so apparent. Small quantities of carbohydrate, however, are transferred to the embryo at this temperature.

These changes led Stokes (26) to suggest that delayed germination was due to nitrogen starvation of the embryo, rather than a need for carbohydrate. This she subsequently proved using embryo culture techniques (27).

Stokes' work has shown that low-temperature treatment does not after-ripen the embryo, but rather has a direct effect on the endosperm. However, she also suggests that some product of embryo metabolism at low temperatures diffuses into the endosperm, bringing about the breakdown of storage protein. She has found that an area of low pH develops in the "mucilage" around the embryo; thus it is possible that excretion of acids by the embryo is responsible for bringing about the changes described. A critical concentration of acids reached after nine weeks of low temperature allowed breakdown to proceed at room temperature. The probability of this suggested mechanism is substantiated by the work of

Eckerson (10), in which it was found that after-ripening of Crataegus embryos was accelerated by treatment with dilute acids.

Stokes' observations (26) on the metabolism of fats in Heracleum do not agree with those of Eckerson (10) and Pack (22). In the after-ripening of Heracleum at 2° C., there was no change in fat content until germination had taken place. Both Eckerson and Pack noted decreases in fat content toward the end of after-ripening. In the latter cases, however, embryos were both after-ripened and germinated at 5° C., so that failure to distinguish between the end of after-ripening and the commencement of germination may account for the apparent discrepancy.

The statement on germination by Toole et al. (31) must be considered critically in the light of findings by Pack (22) and Stokes (26), since it would appear that materials from the endosperm - at least in the cases of Juniperus and Heracleum - contribute greatly to germination. Eckerson's (10) definition of after-ripening, as amended, would still be reasonable, since it appears likely that changes in the embryo are responsible for the initial breakdown of materials in the endosperm.

Tukey (32) considered dormancy as being due to the storage of reduced materials in the embryo or to a balance involving a high proportion of reduced materials. He stated

that after-ripening processes are those involving oxidation and the altering of this balance.

Thornton (30) studied the effect of high and low temperatures on after-ripening of Cocklebur, Xanthium canadense, Mill., in the presence and in the absence of oxygen. He concluded that moist storage at high temperature in the presence of a normal oxygen pressure merely augmented the dormant condition in the majority of cases, because the hydrolizing system remained active over and above the oxidation system. Such a condition resulted in the accumulation of inhibiting substances which produced secondary dormancy. Low-temperature storage, on the other hand, not only altered the type of hydrolysis, but retarded the accumulation of inhibiting substances in the embryo so that metabolic activity was allowed to proceed normally.

Eckerson, according to Thornton (30), had observed the presence of acetaldehyde in dormant Crataegus seeds, and had noted its disappearance when dormancy was broken and growth initiated. Commenting on Eckerson's work where low-temperature storage initiated a change in the pH of the embryo and surrounding tissue, Thornton (30) states, "from these results it is possible that the oxidizing system may be inhibited, thus accounting for the accumulation of acetaldehyde and the primary dormancy. With prolonged storage at low temperature, the inhibiting substances are finally eliminated by a process of slow oxidation, whereby such substances as acetaldehyde are converted

to acids and from there to various end products, so that growth is permitted and at the same time aided in getting underway."

According to Thornton (30), Eckerson found that a temperature of 24° C. resulted in the rapid breakdown of the protein in wheat kernels to polypeptides and then very slowly to amino acids. The embryo at this temperature is surrounded by a mucilagenous substance which apparently causes reduced oxidation, resulting in the production of a small, weak plant. At a temperature of 10° C., however, starch is rapidly hydrolized to sugars and proteins are broken down slowly to amino acids without any detectable sign of polypeptides. The resulting seedling growth is rapid and a sturdy plant is produced.

Stokes (27) observed a predominance of alanine when the seed of Heracleum sphondylium was exposed to a temperature of 15° C., but the accumulation of argenine and glycine at the optimum temperature of after-ripening, 2° - 5° C. She was subsequently able to show that argenine and glycine greatly benefited embryo growth, whereas alanine was not beneficial.

The effect of amino acids as inhibitors of growth is illustrated by the work of Washburn and Niven (33) with Streptococcus bovis. Argenine, at concentrations of 5 mg. per 10 ml. in the medium, provided sufficient amino nitrogen for growth of the organism; but when certain other amino acids or combinations were added, growth was retarded or completely inhibited. Reversal of this inhibiting effect could be achieved

through the addition of certain amino acids at concentrations less than those of the inhibiting agent. It was also noted that the presence of argenine in the medium strongly affected the concentration of the inhibiting acids needed to prevent growth. These authors have suggested that certain amino acids may block the synthesis of others vital for growth.

Audus and Quastel (3) investigated the effect of low concentrations of certain amino acids and amines on the growth of cress (Lepidium) seedlings, and observed inhibiting effects varying from 0% to 80%.

Sanders and Burkholder (23) studied the effect of various amino acid mixtures on the growth of Datura embryos, and found that the growth stimulus from the greatest combination of acids used resulted, not from mere summation of effects of individual acids, but rather from physiological interactions.

Spoerl (24) compared the effect of nineteen amino acids individually with ammonium nitrate and no nitrogen on the growth of Cattleya embryos in pure culture. With very young embryos, from unripe seeds, only argenine supported good growth. All other amino acids used inhibited growth under one or more of the experimental conditions. The inhibiting effect seemed to be greater on germination than on later development. With older embryos, from ripe seeds, aspartic acid acted as a good nitrogen source. Glutamic acid, on the other hand, did not

support good growth, but neither did it inhibit. All other acids used inhibited growth under one or more conditions.

Inhibiting Substances Causing Dormancy

Evenari (11) listed the natural germination inhibitors, that have been identified, as ammonia, hydrogen cyanide, ethylene, essential oils, mustard oils, aldehydes, alkaloids, unsaturated lactones, and unsaturated acids. To this list, Toole et al. (31) added dehydracetic acid, desacetyle, and phthalids.

Evenari (11) states that inhibition from many of these substances may, under certain circumstances, be accompanied by stimulation of germination and growth. This may be due to the intrinsic nature of the substance -- viz., possessing the action of poisons and hormones; to the presence of two substances; or to the transformation of the inhibitor into a stimulator by a slight chemical change during germination.

Moscheov, according to Evenari (11), found that the water extract of wheat roots stimulated the growth of wheat seedlings after an initial inhibition which lasted for three or four days. The same worker was able to eliminate the inhibiting action of the extract by heat, but found that the stimulating action remained. Evenari stated "that these facts point to the existence of

two
/ different substances which are present at the same time in the undiluted juices."

Konis, according to Evenari (11), noted that tomato juice at sufficiently high dilutions will stimulate germination and growth.

Konis and Ullman (18) reported that the inhibiting action of tomato juice can be increased by boiling at 100° C. Heating to 60° C. has a similar effect, only the magnitude is not nearly so great. These authors believed that tomato juice contains, not only an inhibitor, but a stimulating substance as well; thus they explained the heat effect by saying that the stimulating substance is comparatively resistant to a temperature of 60° C., but is destroyed by boiling at 100° C. They have also found that the stimulating effect of tomato juice is more pronounced in dialized juice, and have concluded that the inhibiting factor must be a non-colloidal substance.

Ruge, according to Evenari (11), favored the theory that inhibitors are transformed to stimulators by slight chemical changes during germination. Working with dry fruit of Helianthus, he found that all its parts contained only inhibitors. When the different organs were isolated from seeds following imbibition of the intact seeds for six hours, the plumule and radicle stimulated; the cotyledons and fruit-coat inhibited initially, but after some time the inhibiting action of the

cotyledons was reversed into stimulation. After an imbibition of 15 to 18 hours, all organs stimulated, including the fruit-coat which showed an initial inhibition.

Evenari (11) states that Ruge, Veh and Soding have developed an interesting theory about the formation of these inhibitors and the transformation of them into stimulators. The following steps supposedly are involved:

(a) Hydrolysis of a nitril-glucoside of the amygdalin type and the formation of HCN, a known inhibitor.

(b) Addition of elemental sulphur to the HCN molecule by the action of rhodanase and formation of HSCN, which is a stimulator.

According to Barton (7), many authors have found auxins in seeds, and some have shown that the auxin content decreases during germination.

Barton (6) carried out comprehensive tests in which many different kinds of quickly germinating seeds were treated with low concentrations of synthetic plant-growth substances in vapour, solution and dust forms. In general, the results showed that neither the rate at which seeds germinated nor the percentage germination was influenced by the treatments.

Working with seeds which normally exhibit dormancy, Barton (7) also found no beneficial effect of treatment with growth substances. When concentrations of materials greater

than those normally found in seeds were used on fully after-ripened material, the latter were thrown into a period of secondary dormancy which she was able to remove by low-temperature treatment.

Grace (17), on the other hand, has shown that seed treatment of grain with synthetic growth substances produced a marked stimulation effect.

Evenari (11) stated that if auxin present in seeds were to function as a hormone affecting germination, then the action of inhibitors could be explained by either auxin destruction or inactivation.

Thimann and Lane (29) have shown that the response of roots to auxin gives an optimal curve which differs from the response of shoots only in that the peak of the curve for roots is at an exceedingly low concentration, while the peak for shoots is close to the concentration which causes damage -- i.e., the difference is quantitative but not qualitative.

Thimann (28) found that when Avena roots were inhibited by auxin for a day or so and then transferred to water, their subsequent growth was hastened and eventually exceeded that of the controls. He has stated that these after-effects were probably controlled by several factors; but later (29) presented evidence to show that the auxin in the root was destroyed

and that its concentration fell until it reached an accelerating level.

Larsen (19) found that the growth inhibitors, parasorbic acid and anemonin, were able to mask the effect of 3-indole-acetic acid in the Avena coleoptile test. Although these materials are considered to be antagonistic substances to auxin, the Avena coleoptile test is not the appropriate one to use in this case. A true antagonist to auxin will retard growth of shoots and promote the growth of roots.

Juel, according to Larsen (19), applied the growth-retarding substance from tomato fruit to growing roots of pea seedlings; at all concentrations, he found inhibition of growth and concluded that the growth-inhibiting substance was not an antagonist to auxin.

Growth-promoting substances are thought to play a part in the after-ripening of apple-seed embryos; yet some diversity of opinion is apparent from observations made.

Luckwill (20) found that a growth-promoting substance appeared in the after-ripened embryos of apple seeds just prior to germination. He stated that this did not show up in dry storage. Keil, according to Evenari (11), presented a theory concerning an inhibition/stimulation mechanism in these seeds involving both HCN and heteroauxin. Failure of the seeds to germinate was attributed to the presence of HCN in the tissue

surrounding the embryo. This material narcotizes all the cells and lowers their response to heteroauxin, which is not attacked by HCN. When the inhibitor is removed, the seeds germinate, for narcosis of the cells to heteroauxin is abolished.

Epicotyl Dormancy and After-ripening

Barton studied germination in the seeds of Lilium (5) and Paeonia (4), two genera having species which show epicotyl dormancy. In both cases, she found embryos required high temperature for germination, followed by a period of low temperature to after-ripen the epicotyl. In no instance was there any indication that epicotyls might after-ripen at the temperature of germination.

Giersbach (16) studied seedling production in ten species of Viburnum and found all but two in need of special treatment for after-ripening the epicotyl. As with Lilium and Paeonia, the eight species with epicotyl dormancy responded to high temperature for germination, but required a critical low-temperature period for epicotyl after-ripening.

Flemion (12) studied the growth of excised non-after-ripened embryos of seeds from a number of plants in the family Rosaceae. Seedlings were produced in most cases at room temperature, but all were dwarfs. These dwarfed seedlings exhibited sufficient root growth to maintain normal tops when the

latter were grafted to them. When reciprocal grafts were made, however, the normal roots had no effect on the growth of the dwarfish tops. It would appear from this that the factor causing dwarfishness is located in the tops.

Removal of the cotyledons in fully after-ripened seedlings had little effect on the growth of the epicotyl other than to result in the production of a less vigorous plant. The treatment, however, failed to produce a dwarfed seedling.

Barton and Schroeder (8) found that seeds of Convallaria majalis, L., were not receptive to low temperatures for after-ripening the epicotyl until a certain growth stage had been reached. This was indicated by growth of the shoot to the point where it had broken the cotyledonary sheath. Five months were then required to after-ripen the epicotyl, following which light was needed for the production of green shoots.

MATERIALS AND METHODS

(a) Physical and Chemical Nature of the Seed

Studies, preliminary to the investigation of dormancy, were made on embryos and endosperms of seeds of the 1955 crop, obtained from Herbst Bros., Seedsmen, New York City, in the spring of 1956.

Measurements of embryo length were made using a microscope fitted with a filiar eye-piece. Endosperm tissues were sectioned using a freezing microtome, and were treated with potassium iodide and with Sudan III.

Oil and nitrogen determinations were made on endosperm material from dry seeds, and from soaked seeds which had been in the 20° C. germinator for 30 and 60 days. It had been the original intent that that these studies would be carried out periodically during the germination period; however, erratic behaviour of the seeds to germinative conditions resulted in this phase of the work being abandoned after 90 days.

Oil extractions were made with petroleum ether, using the official method outlined by the A.O.C.S. (1) for cottonseed. Total nitrogen was determined on fat-free residues, using the microkjeldahl method described by the A.O.A.C. (2), except that the one-hour digestion period as outlined by Galston and Dalberg (15) was used.

Soluble nitrogen was extracted from 600 mgm. samples of fat-free material in 120 ml. of distilled water by shaking continuously for 16 hours and then filtering to remove the residue. Two ml. of the Galston and Dalberg (15) digestion mixture were added to 2 ml. of the extract, and the one-hour digestion was carried out prior to distillation.

(b) General Procedures Employed in Germination Studies

Fruit was harvested on October 15, 1956, from plants growing on the University of Alberta Campus. Seeds were extracted from the pulp by crushing the fruit. In no instance was the normal fermentation method of extraction employed because of the danger of introducing a soaking effect. All seeds were rinsed thoroughly in a stream of tap water following extraction, and where whole seeds were required for treatment, planting followed immediately. In the case of treatments involving decorticated seeds, extractions from the fruit were not made until just prior to the time of endocarp removal. Thirty-seven hundred seeds were used in the experiments, and of these 1,900 were decorticated. Extraction, decortication and planting were completed in a week.

Seeds were planted in shallow waxed paper containers of 135 ml. capacity. These were found to be convenient for germination studies because of their depth, durability and reasonable cost.

The following planting procedure was adopted: Eighty ml. of vermiculite were tamped firmly into the bottom of each container. This was moistened and 25 seeds were placed in rows on the surface of the tamped material. The container was then filled to the top with vermiculite, covering the seeds to a depth of one-quarter inch. Tap water was added carefully until the contents of each container were saturated, and excess water was poured off. The containers were placed in controlled temperature chambers and kept there for the duration of the experiments or transferred as required. The germination medium was soaked every seven days, and seeds were examined periodically to record germination.

To facilitate examination of the seeds, the contents of each container were dumped into a box sieve having a one-eighth-inch mesh. Water was carefully forced through the material to remove the vermiculite. Following examination, the seeds were re-planted in fresh vermiculite, watered and returned to the appropriate temperature chamber.

(c) Temperature Treatments

The effects of three constant temperatures and three sets of alternating temperatures on germination of whole and of decorticated seeds were studied over a period of 104 days. One hundred seeds constituted a sample in all cases.

The following temperature treatments were provided:

1. Constant 2° C.
2. " 5° C.
3. " 20° C.
4. 2° C. alternating daily with 20° C.
5. 5° C. " " " 20° C.
6. 2° C. " weekly with 20° C.
7. 5° C. " " " 20° C.
8. 2° C. " bi-monthly with 20° C.
9. 5° C. " " " 20° C.

After 110 days, all the samples were transferred to the 20° C. chamber and maintained for 46 days, when germination counts were again made.

(d) Soaking Treatments

Soaking, prior to sowing, is a common horticultural practice with those seeds which absorb water freely. Although the endosperm of V. trilobum does not imbibe water in the early stages of germination, the effect of soaking might be advantageous. The endocarp is permeable to moisture; therefore, the soaking of whole seeds could result in quick and efficient hydration of the testa, which might otherwise offer some restriction to oxygen.

The effects of soaking treatments were studied on the germination of whole seeds, which were subsequently planted and maintained at 20° C. Three samples of 75 seeds each were shaken continuously in tap water for 3-hour, 24-hour and 48-hour periods prior to planting.

Because material from fresh seed diffuses into tap water on soaking, the liquid was changed at 12-hour intervals during the course of the 24-hour and 48-hour treatments. The liquid from each treatment was saved for further study. Since 150 ml. of final solution were required, and since standard volumes of water were needed for each soaking, the following schedule was used:

Treatment	No. of changes	Vol. of soaking liquid	Subsequent handling
3 hr.	0	37.5 ml.	Made up to 150 ml.
24 hr.	2	37.5 ml.	Combined and made up to 150 ml.
48 hr.	4	37.5 ml.	4 extracts combined

(e) Rinsing Decorticated Seeds

The possible beneficial effect of soaking on hydration of the testa was considered in the preceding section. Should an inhibitor be present in either the endocarp or the

testa, however, the effect of soaking conceivably could be deleterious rather than advantageous. In order to provide the beneficial effects of hydration to the testa and at the same time eliminate the effect of a possible inhibitor, 400 decorticated seeds were rinsed prior to being placed in the germination medium at 20° C.

In the course of rinsing, seeds were placed in a flask and swirled in fresh tap water for a period of one minute. The liquid was then replaced with fresh water and the swirling action repeated. This procedure was carried out eight times prior to planting. Germination from this sample was compared with that from the 400-seed samples of whole and of unrinsed decorticated material.

(f) The Effect of Seed and Endocarp Extracts on the Growth of Test Material

Water extracts from whole seeds of the 1956 crop (Section d), as well as from air-dried endocarp material of the 1955 and 1956 seed crops, were tested on seeds and seedlings of the wheat variety Canus (Triticum vulgare), on seeds of the flax variety Redwing (Linum usitatissimum), and on seedlings of V. trilobum.

In order to determine whether or not an inhibiting substance is removed from V. trilobum during soaking, wheat samples consisting of 90 grains were immersed for a period of 16 hours in each of the extracts from the treatments of Section (d).

Following the immersion period, 60 grains were selected from each of the samples and planted on vermiculite, 20 seeds per container, according to the method described in Section (b). The vermiculite was then moistened with what remained of the water extracts and the containers were placed in a germination cabinet which maintained high humidity and a temperature of 26° C.

Root measurements were taken at the end of seven days and comparisons made with check lots of wheat which had been immersed for 16 hours in tap water.

To determine the effect of extracts of endocarp material alone, a further study was conducted. Endocarps from both the 1955 and the 1956 crops were air-dried and ground in a Wiley mill to a fineness that would pass a No. 20 screen. One 3-gram sample, representing the endocarps from approximately 100 seeds, was taken from each seed lot. Each sample was extracted in 450 ml. of water on a mechanical shaker.

At the end of three hours, supernatants from each extraction were removed and replaced with 450 ml. of fresh water. Shaking was repeated for a further 21 hours. Following removal of supernatants at the end of this period, 450 ml. of fresh water were again added and shaking continued for a further 24-hour period.

Eight 50 ml. aliquots from each of the three extracts were taken. Four of these were used to soak samples of

wheat; and four to soak the samples of flax. Thirty seeds were soaked in each aliquot.

Test materials were allowed to remain in the extracts overnight, after which 20-grain samples were selected from the wheat and 10-seed samples from the flax. These were planted on vermiculite according to the method outlined previously. The medium in each case was moistened with the solution in which each sample had been immersed. The seeds were then transferred to the 26° C. germinator. Measurements on total root length were recorded seven days later.

In another set, a more concentrated extract from the endocarp was tested for the effect upon growth of roots of wheat and Viburnum seedlings. A 48-hour extraction of 3.0 gm. of dried ground material from 1956 seed was made using 100 ml. of water.

The wheat was germinated by being placed crease side down on moist paper towelling in a humid germination cabinet which maintained an air temperature of 26° C. After 24 hours, it was possible to select uniform samples of seedlings. In all cases, the radicle had emerged and was just touching the towel.

Viburnum seedlings were selected from unrinsed decorticated seeds which had recently germinated. The mean length of the radicle was 5 mm. Three samples of wheat and two of Viburnum, each containing 90 seedlings, were placed on filter

paper in petri plates, 30 seedlings to a plate. In the case of wheat, three treatments were used. These consisted of two concentrations of the extract, the original and a 1:100 dilution along with a tap-water check. Only two treatments were applied to Viburnum because of a scarcity of seedlings. In this case, the effects of the original extract and the tap-water check were compared. In all cases, 10 ml. of liquid were used with 30 seedlings.

Root measurements were made on wheat after the material had been in the 26° C. germinator for 24 hours. The Viburnum seedlings were allowed to remain in the germinator for 10 days before measurements were taken.

(g) Epicotyl Dormancy and After-ripening

Preliminary experiments dealing with seedling production have shown that epicotyl dormancy can be completely overcome on exposure to light at room temperature if the seedlings have been previously exposed to 5° C. for three months.

In order to gain further information on the temperature requirements of the epicotyl for after-ripening, an experiment was conducted to observe the visual effects of two temperature treatments on the process. Seedlings from decorticated seeds were used for this study.

Two lots of seedlings were transplanted to pots containing vermiculite when the radicles had reached a minimum length of one and one-half inches. One lot was exposed to 5° C., while the other was maintained at 20° C. Samples of these seedlings were brought into constant light at room temperature at bi-monthly intervals. A third lot was selected for transfer to light as soon as the seedlings had attained the mimimum root length.

Thirty seedlings from each of the three lots were exposed to light at any one time, and were placed in Erlenmeyer flasks of 300 ml. capacity on moist, non-absorbent cotton islands floating in water. Three flasks were used for each treatment, ten seedlings being placed in each. Light was supplied by two 16-Watt fluorescent tubes held in a porcelain reflector eight inches above the tops of the flasks. This source of light provided an intensity of 300 foot candles.

Since the endocarp and the endosperm frequently adhere to the cotyledons in the course of epigeous germination, the design of the experiment was extended to determine the effect of the adherent endosperm on the behaviour of the seedling following its exposure to light.

To test this, endosperms were removed with a pair of surgical scissors from samples A, C, E, G and H just prior to light exposure. To give greater scope to the combined experiments, endosperms were removed from seedlings in treatments B,

D, F and I after these had been exposed to light for two weeks. All treatments are summarized in the following:

Post-germination treatments

	Duration of dark storage preceding		Light treatment at room temperature
	at 5° C.	at 20° C.	Time of cotyledon exposure
A	2 wks.		Immediately
B	2 wks.		After two weeks
C	4 wks.		Immediately
D	4 wks.		After two weeks
E		2 wks.	Immediately
F		2 wks.	After two weeks
G		4 wks.	Immediately
H			Immediately
I			After two weeks

The combined experiment provides the means for testing time and temperature effects throughout, and temperature and light effects between A, B, C and E, F, G. In addition, sample B is comparable to sample C, and sample F to sample G, in the respect that the endosperm remained on the seedlings the same length of time.

To provide controls for both post-germination temperature treatments, samples H and I were exposed to light immediately after the radicles of the seedlings had reached the minimum length of one and one-half inches.

During the course of the foregoing experiment, one additional aspect of the problem was studied. This was concerned with the effect of cotyledons on after-ripening of the epicotyl. Two samples of 30 seedlings each, one of which had been held at 5° C., the other at 20° C., for two weeks, were transferred to light following removal of the endosperms. After two weeks' exposure to light, the cotyledons were completely removed from half the seedlings. At this time, all the cotyledons were a deep red color, with no sign of chlorophyll present. The epicotyls were barely discernible following excision of the cotyledons. These seedlings were maintained in continuous light for observation.

RESULTS AND DISCUSSION

The seed of Viburnum trilobum consists, very largely, of a hard white endosperm. Cells of this tissue are invacuolate in dry seed and show a negative reaction to the iodine test for starch. All cells, however, stain with Sudan III, indicating the presence of abundant fat.

The embryo is minute, but not rudimentary, and is embedded in the endosperm close to the hilum in the cuspidate tip of the seed (Figure 1). Cotyledons are spathulate-oblong and about as long as the stout obtuse radicle, although slightly broader. Measurements on the basis of a 20-seed sample show the mean length of the embryo to be .72 mm., roughly one-seventh the length of the seed.

The outer seed covering is a hard chitinous, moisture-permeable endocarp, which, though quite flexible when wet, is brittle when dry. The seed conforms to the interior of the endocarp and is covered by a thin adherent testa.

The results of both oil and nitrogen determinations carried out on the endosperm prior to and during the first two months' exposure to germinative conditions are given in Table 1.

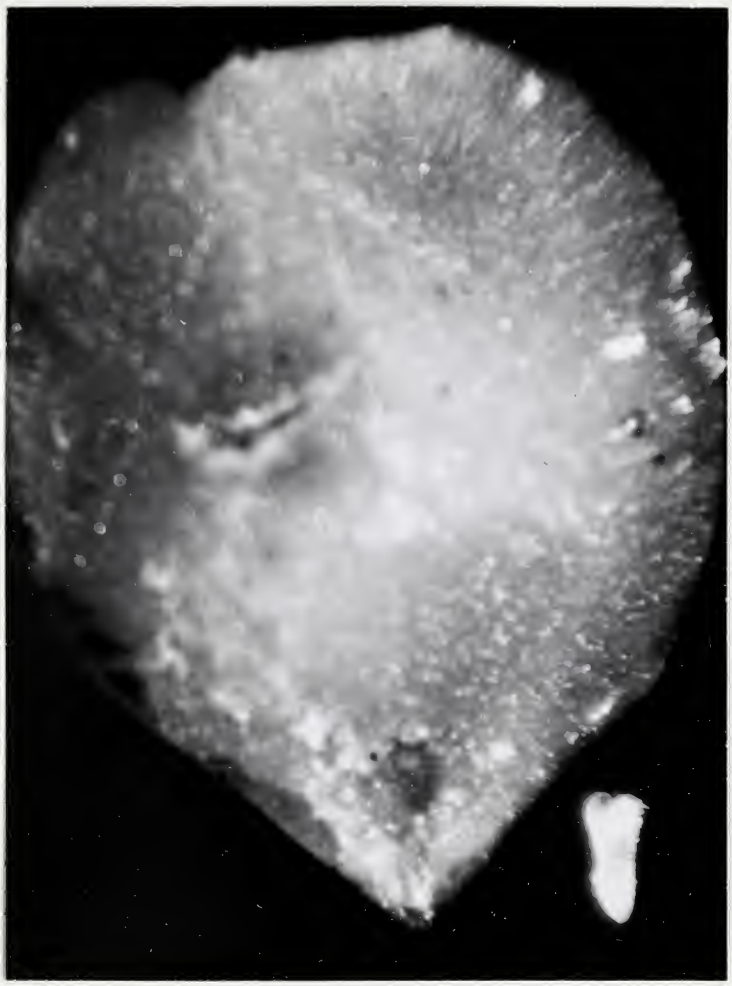

Figure 1. The size relationship of endosperm to embryo in freshly harvested seed of V. trilobum. (Magnification, X25)

TABLE 1

Percentage changes in endosperm reserves during storage of seed under germinative conditions at 20° C.

Fraction	Fresh seed	30 days	60 days
Oil *	25.17	23.52	19.43
Total nitrogen **	3.89	3.84	3.85
Soluble nitrogen **	1.19	1.64	.80

* Expressed as % oven dry weight.
** Expressed as % oil-free residue.

It will be seen from these data that the first notable change in the endosperm was an increase in soluble nitrogen observed following 30 days' exposure to germinative conditions. Fat metabolism apparently did not begin until sometime later, as there was very little decrease in this fraction until the sixtieth day. This was accompanied by an even greater decrease, proportionally, in soluble nitrogen. No apparent decrease in total nitrogen accompanied this latter change, indicating that the nitrogenous materials were not being transferred to the embryo.

Erratic behaviour of these seeds to germinative conditions at 20° C. made it impossible to conduct a reliable investigation of changes in the endosperm up to the time of germination. Thus, although it appears likely that the embryo has a need for food material from the endosperm before germina-

tion can take place, there is also the possibility that the embryo must first be put into a responsive condition by an appropriate after-ripening treatment.

From Table 2, the favorable effect of germinative conditions at 20° C. on the germination of seeds from the 1956 crop is quite noticeable during the first 80 to 90 days; a marked decrease in rate of germination, however, may be seen with increased exposure to these conditions.

TABLE 2

Germination of freshly harvested, intact and decorticated *V. trilobum* seeds in response to temperature

Temperature treatment in degrees C.	% Germination									
	Intact seeds					Decorticated seeds				
	Days					Days				
	50	66	80	90	104	50	66	80	90	104
2°, constant	0	0	0	0	0	0	0	0	0	0
5° "	0	0	0	0	0	0	0	0	0	0
20° "	0	6	16	30	30	31	42	47	47	47
2° - 20°, daily	0	0	0	0	0	0	2	2	2	2
2° - 20°, 7 days	0	0	0	0	1	0	0	0	0	0
2° - 20°, 14 days	0	0	0	0	0	0	0	0	0	0
5° - 20°, daily	0	0	0	0	0	0	0	0	0	0
5° - 20°, 7 days	0	0	0	0	0	0	0	0	0	1
5° - 20°, 14 days	0	0	0	0	0	0	0	0	0	0

The increase in germination due to endocarp removal is also of interest here. Giersbach (16) noted no such effect with Viburnum acerifolium L., V. dentatum L., V. dilitatum Thunb., V. lentago L., V. opulus L., V. prunifolium L., or V. rufidulum Raf., when endocarps were removed or scarified with acid. The germination of V. trilobum was not studied by this author; therefore, no entirely satisfactory explanation for the difference in behaviour shown by the other species can be offered. In these studies, counts made 52 days later (Table 3) show a general increase of 19% germination with removal of the endocarp. This difference is statistically significant.

It will be seen from Tables 2 and 3 that the effects of alternating temperatures on germination did not become apparent until after the seeds had been transferred to constant 20° C. temperature for several weeks. Analysis of these data revealed that the effect of the temperature treatments was statistically significant.

The effects of daily, weekly and bi-monthly alternations of temperature, as well as that of constant 20° C., were significantly better than the effect of the constant 2° C. and 5° C. temperatures.

For intact seeds, the effect of daily alternations was significantly better than that of the bi-monthly alternations. No such difference was apparent in the decorticated

TABLE 3

Germination of V. trilobum after 110 days' treatment followed by 46 days at 20° C.

Temperature treatment in degrees C.	Germination percentages after 46 days at constant 20° C.		
	Intact seeds	Decorticated seeds	Average for temperature treatments
2°, constant	4	20	12
5°, "	4	20	12
20°, "	36	63	50
2° - 20°, daily	75	83	79
2° - 20°, 7 days	59	97	78
2° - 20°, 14 days	47	96	72
5° - 20°, daily	91	80	86
5° - 20°, 7 days	86	89	88
5° - 20°, 14 days	60	82	71
Mean	51	70	
L.S.D. at 5% - seed type x temperature	22		
temperature			21

seeds. This differential response of seed type to temperature is reflected in the statistically significant interaction.

In discussing Table 3, it should be mentioned that these data represent the sixth examination of the seeds for germination. Thus, the interpretation of the temperature effect at 156 days is complicated by the effect of the five previous rinsings, which might have effectively removed any soluble germination inhibitor if one were present.

The foregoing observations suggest that the factor or factors inhibiting germination are not completely removed by temperature treatments alone.

The effect of soaking on germination is shown in Table 4. These treatments appear to have some beneficial effect on germination at 20° C. when compared with unsoaked check materials.

TABLE 4

Effect of soaking treatment on germination of intact seeds at 20° C.

Treatment	% Germination			
	Days			
	50	66	88	94
Soaking 3 hrs.	0	3	12	13
" 24 hrs.	0	4	20	27
" 48 hrs.	0	8	15	20
Unsoaked	0	0	4	8

Some explanation for the favorable effect of soaking on germination is evident from the results obtained when the extracts from the soaking treatments were used on test samples of wheat (Table 5). The extract obtained after 24 hours' soaking had a greater inhibiting effect on the growth of wheat roots than either of the other two, and the difference was statistically significant. A comparable increase in germination (Table 4) over that of the other two treatments was also obtained with seeds which had been soaked for 24 hours. This suggests that increased germination following soaking is at least partly due to the removal or the dilution of an inhibiting substance.

TABLE 5

Effect of extracts from soaking experiment on growth of wheat roots

Extract	Average root length in cms. 1 wk. after treatment
Water check	34.8
3-hour extraction	30.1
24-hour "	24.4
48-hour "	30.1
L.S.D. at 5%	5.4

The striking effect of rinsing decorticated seeds on the rate of germination may be seen in Table 6. These results indicate that the substance responsible for inhibition

is localized on or within the testa, and that its presence is associated with failure of the seeds to respond to germinative conditions.

TABLE 6

Effect of endocarp removal with and without rinsing of testa on subsequent germination of V. trilobum

Treatment	Percent germination at 20° C.						
	Exposure in days						
	45	50	55	66	80	94	104
(Check) Intact seed	-	1	-	12	22	29	32
Endocarp removed; testa not rinsed	-	18	-	29	42	51	52
Endocarp removed; testa rinsed	41	52	57	59	59	59	-

It will also be noted that the seeds from which this substance had been removed showed a decline in the rate of germination similar to that exhibited by the seeds of the other two treatments. This suggests that the mere removal of an inhibitor does not account for the high germination resulting from treatments described in Table 3. Evidently, the high germination percentages shown in Table 3, particularly those in the case of decorticated seeds, resulted from a combination of factors: namely, the removal or the dilution of an inhibitor and the use of alternating temperatures prior to exposure at 20° C.

Although the data of Table 6 point to the existence of a water-soluble inhibitor in or on the testa of Viburnum trilobum, it cannot be concluded from this that the material responsible for inhibition is localized solely in this structure. In fact, the data of Table 7 suggest the presence of an inhibiting material in the endocarp as well. The inhibiting effect of all the endocarp extracts is apparent in wheat, but only the effects obtained from the use of the primary extracts in each lot and the secondary extract from 1956 material were statistically significant. These results, with wheat as the test material, suggest that a decrease in inhibition is due to dilution of the inhibitor.

While the use of endocarp extracts on flax gave significant results in all cases, inhibition occured only when the primary extraction from 1956 material was used. All other extracts produced stimulation effects. These results suggest that dilution of the inhibiting material beyond a certain critical point results in stimulation, after which increased dilution gives decreased stimulation.

With flax, there is no evidence to suggest that dilution up to the point where stimulation was first noticed would result in anything but decreased inhibition. Had dilutions intermediate with those causing inhibition and stimulation been made, however, it is quite likely that both decreased inhibition and low stimulation values would have been encountered in this range. Such effects on root growth have been recorded

TABLE 7

Effects of endocarp extracts from 1955 and 1956 V. trilobum seeds on growth of wheat and flax roots

		Average root length in cms. one week after treatment	
		Wheat	Flax
Water check		42.3	2.4
Extracts from 1956 seeds	1. First 3 hours	30.0	1.9
	2. Next 21 hours	36.3	4.9
	3. Next 24 hours	40.5	2.9
Extracts from 1955 seeds	1. First 3 hours	33.6	3.8
	2. Next 21 hours	40.5	3.4
	3. Next 24 hours	41.7	2.8
L.S.D. at 5%		5.5	0.1

by both Evenari (11) and Thimann (28).

The difference in response of wheat and flax to the same extracts is probably due to inherent differences between the two genera. Wheat has a starchy kernel, while flax has an oily seed. Therefore, the normal metabolic processes in such seeds, both before and subsequent to germination, might be quite different.

The effects of endocarp extracts from Viburnum seeds of the 1956 crop on the growth of wheat and Viburnum seedlings are shown in Table 8.

TABLE 8

Effects of endocarp extracts from a 48-hour extraction on root growth of wheat and Viburnum seedlings. (Concentration of original extract - 30 mg. per ml.)

Extract	Root length in mm.	
	Wheat	Viburnum
Original	2.8	10.8
1/100 dilution	14.1	--
Water check	13.0	23.5
L.S.D. at 5%	.9 mm.	"t" significant at 5%

The results show that the use of the original extract causes an inhibition of root growth on both lots of seedlings. This effect is statistically significant. The use of the 1/100 dilution also gives a statistically significant effect; however, the result is stimulatory.

Considering the data on germination of Viburnum (Table 6), and those on root growth of the seedlings (Table 8),

it is now evident that there are one or more soluble substances in the seed which inhibit both germination and root elongation.

Giersbach (16) has shown that a temperature of 5° C. was required for the after-ripening of dormant epicotyls in all of the northern forms of Viburnum which she studied. From the data of Table 9, it can be seen that the epicotyls of Viburnum trilobum will after-ripen at both 5° C. and 20° C.

TABLE 9

Effect of treatment of Viburnum seedlings on shoot production in light (30 seedlings per treatment)

Sample	WEEKS IN DARK prior to being transferred to light		Number of seedlings with shoots				
			Days in light at room temperature				
			30	40	45	50	55
A	at 5° C.	2	0	11	11	12	15
B		2 *	0	3	8	10	12
C		4	2	8	12	15	20
D		4 *	4	12	13	15	17
E	at 20° C.	2	1	4	8	8	10
F		2 *	0	9	10	10	12
G		4	3	12	14	15	15
H		-	0	0	0	0	3
I		- *	4	4	5	7	10

* Samples from which endosperm was removed 2 weeks following transfer to light.

It will be observed from these data, however, that epicotyls from seedlings which had not received continued temperature treatment in the dark (H) gave exceedingly poor shoot production if the endosperms were removed from the seedlings just prior to light exposure. On the other hand, if the endosperms were not removed until two weeks after transfer to light, shoot production was enhanced. When seedlings from which the endosperm had been removed were transferred from dark to light, the color of the cotyledons of those seedlings which had been held at 20° C. changed from pink to dark red, usually during the first 48 hours. Cotyledons from seedlings which had been held at 5° C. were already dark red at the time the endosperms were removed.

Since it had been observed during the preliminary work that seedlings with red cotyledons gradually attained normal green pigmentation prior to shoot production, it was felt that the persistence of the red color might be associated with failure of the epicotyl to after-ripen. Further observations were therefore made on the behaviour of the cotyledons during the course of the light treatments.

A comparison of the data from Tables 9 and 10 shows that normal green pigmentation of the cotyledons is not necessarily associated with after-ripening of the epicotyl. The cotyledons of seedlings in sample H are completely green within seven days; yet the first shoot production was not recorded until 48 days later. Samples B and D, on the other hand, produced shoots from three

and 12 seedlings, respectively, 26 days after the cotyledons were exposed to light; yet no greening of cotyledons was noticed prior to 55 days in light.

It may be seen from Table 10 that the seedlings of sample H, on which the endosperm had been retained for the least time, required about two weeks' less exposure to light for chlorophyll formation. This suggests that with prolonged assimilation of the products of the endosperm the appearance of chlorophyll in the cotyledons is delayed.

TABLE 10

Effect of treatment on the greening of cotyledons in light

Sample	DARK TREATMENT	Weeks	Exposure of cotyledons, in days, to effect complete removal of red pigment
A	at 5° C.	2	25
B		2 *	--
C		4	45
D		4 *	--
E	at 20° C.	2	20
F		2 *	35
G		4	20
H		-	7
I		- *	35

* Samples from which endosperm was removed 2 weeks following transfer to light.

The inhibiting effect of the cotyledons on epicotyl growth (Figure 2), or on epicotyl after-ripening, is apparent from the data of Table 11. Even after two months' exposure to light, epicotyl development did not occur in more than 50 percent of the seedlings possessing cotyledons.

TABLE 11

Effect of cotyledon removal on growth of Viburnum epicotyls from two samples of seedlings previously stored in darkness at 5° C. and 20° C.

Dark treatment preceding cotyledon removal	Light treatment at room temperature	
	% epicotyls growing after 10 days	
	Seedlings with cotyledons off*	Seedlings with cotyledons intact
2 wks. at 5° C.	100	2
2 " " 20° C.	100	0

* Cotyledons removed after seedlings in light for 2 weeks.

The immediate growth response of both samples of seedlings when cotyledons are removed supports the observation made earlier that no marked differences in effect from storage at either 5° C. or 20° C. can be observed on shoot production following exposure to light. Contrary to this, however, Giersbach (16) reported a marked difference in response of the epicotyl after the seedlings were exposed to the same two temperatures for several months. No explanation for this be-

Figure 2. Effect of cotyledon removal on epicotyl growth following 40 days' exposure to light. The two seedlings to the left of centre have cotyledons intact and show no epicotyl growth. Those to the right have had the cotyledons removed and show normal epicotyl development.

haviour can be offered on the basis of observations made with V. trilobum, although seedlings exposed to 5° C. would be expected to retain more carbohydrate than those exposed to 20° C., and thus show more vigorous shoot growth when transferred to light at room temperature.

Three possible explanations for the effect of the cotyledons on epicotyl growth in V. trilobum are:

(1) That a competitive relationship exists between the cotyledons and the epicotyl for food reserves.

(2) That the cotyledons inhibit the epicotyl in a manner similar to that by which the apical bud of more mature plants inhibits the growth of laterals.

(3) That a combination of these two suggested mechanisms is operative.

SUMMARY AND CONCLUSIONS

Dormancy as it is related to seedling production in the American highbush cranberry, Viburnum trilobum, Marsh., has been studied. During the course of the investigation, two distinctly different periods of dormancy were encountered.

Failure of the seed to germinate promptly when exposed to the germinative conditions employed in this study was found to be caused, in part, by the presence of a water-soluble inhibiting substance found present in both the endocarp and the seed. Alternating temperature treatments given prior to constant 20° C. were found to be a further requirement for maximum germination.

The effect of extracts from whole seeds and from dried endocarps was tested on Triticum, Linum and Viburnum. High concentrations were found to have inhibiting effects on root growth of all test plants. Lower concentrations were tested on Triticum and Linum and showed results varying from decreased inhibition to marked stimulation. This behaviour is similar to that attributed to the effect of inhibiting substances found present in many other seeds.

A block to continued growth and development of the seedling was encountered in the epicotyl following germination.

Shoot production was observed in seedlings which had been exposed to constant light for 50 days following storage in the dark at 5° C. and at 20° C. for periods varying from two weeks to one month. No apparent difference in the effectiveness of these two temperatures for after-ripening the dormant epicotyl was shown.

Seedlings which had been stored in the dark at 5° C. and at 20° C. for two weeks after germination showed complete shoot production when cotyledons were removed following two weeks' exposure to light. These observations indicate that dormancy in the epicotyl of the V. trilobum seedling is effected by the cotyledons rather than by an unfavorable post-germinative temperature.

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